

BioScientific Review (BSR)

Volume 3 Issue 1, 2021

ISSN_(P): 2663-4198 ISSN_(E): 2663-4201

Journal DOI: <https://doi.org/10.32350/BSR>

Issue DOI: <https://doi.org/10.32350/BSR.0301>

Homepage: <https://journals.umt.edu.pk/index.php/BSR>

Journal QR Code:



Article:

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Article DOI:

<https://doi.org/10.32350/BSR.0301.02>

Article QR Code:



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To cite this article:

Ghafoor M, Khan MX, Ramzan A, Abbas SH. Health assessment of white-rumped vultures (*Gyps bengalensis*) from Changa Manga, Lahore: A case study. *BioSci Rev.* 2021;3(1):17–24.

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A publication of the
Department of Life Sciences, School of Science
University of Management and Technology, Lahore, Pakistan

Health Assessment of White-rumped Vultures (*Gyps bengalensis*) from Changa Manga, Lahore: A Case Study

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Article Info

Received: Feb 23rd, 2021

Revised: Apr 26th, 2021

Accepted: Apr 28th, 2021

Keywords:

Changa Manga,
Gram staining,
Gyps bengalensis,
parasites

Abstract

Vultures are considered an important indicator of a healthy environment and they play a vital ecological role as scavengers. The current study was designed to estimate the health of the critically endangered *Gyps bengalensis* in Changa Manga, Lahore using a technique that does not require drawing blood. Fecal matter of seven pairs was examined and analyzed for protozoan or helminth infections. Gross examination involved checking the consistency and color of feces, presence of blood in feces and gross parasites. Procured samples had different colors and consistency but no blood was found in them. Pair 1 was infested with oocytes; however, in pairs 5 and 6 only males were infested, while only the female was infested in Pair 7. Pairs 2, 3, and 4 were completely healthy. Gram staining was also performed to assess the presence or absence of bacteria culture in the digestive tract of *Gyps bengalensis*. All pairs were either gram-positive or gram-negative except Pair 5, which was negative for all Gram staining. The current work introduces a technique to assess the health of vultures without drawing blood samples, a process that disturbs their life cycle.

1. Introduction

Pakistan has significant biodiversity and landscape richness. About 670 species of birds have been recorded in Pakistan, including migratory species. Vultures are scavenging birds and play an important role in maintaining the environment. They mostly eat dead or sick animals and thus clean the environment [1, 2]. There are two main groups of vultures, that is, Old World vultures and New World vultures. The New World vulture belongs to the family Cathartidae, order Accipitriformes. Seven species are found in the temperate and warm areas of the Americas. New World vultures have a good sense of smell. Old World vultures are found in Europe,

Africa and Asia. They belong to the family Accipitridae and order Accipitriformes [1, 3].

There are 23 species of vultures in the world. Nine species are critically endangered, three are endangered, and four are near threatened [4]. Nine species of vultures are found in Asia. These include WRV (white-rumped vulture; *Gyps bengalensis*), CV (cinereous vulture; *Aegypius monachus*), HV (himalayan vulture; *Gyps himalayensis*), RHV (red-headed vulture; *Sarcogyps calvus*), EV (Egyptian vulture; *Neophron percnopterus*), GV (griffon vulture; *Gyps fulvus*), SBV (slender-billed vulture; *Gyps*

tenuirostris), BV (bearded vulture; *Gypaetus barbatus*) and IV (Indian vulture; *Gyps indicus*) [5]. Of the above nine species found in Asia, Eight are found in Pakistan (with the exception of). Slender billed vulture.

White-rumped vultures (WRVs) are medium sized vultures. The adults are about 75-85 cm tall with a wingspan of 180-210 cm. Their weight range is 3.5-7.5 kg. The sexes are approximately equal in size. Adults are darker than juveniles, have a white neck ruff, and a blackish plumage [6]. WRVs lay eggs twice a year but the egg hatching ratio is once a year [7]. Their name is derived from the white patch of feathers on their lower back and upper tail. These vultures are endemic to South Asia and remain critically endangered in Pakistan. Their population has declined by over 95% since the early 1990s [8]. The cause of its rapid decline is the veterinary use of the non-steroidal anti-inflammatory drug (NSAID) diclofenac. It is used in domesticated animals as a pain killer [9]. This decline has resulted in a decrease of WRV population to 0.1% of its original size [10-12]. The loss of population has caused major secondary impacts including the increasing number of feral dogs that exploit the vultures' food sources. Gyps Vulture Restoration Project is run by

WWF-Pakistan. The center piece of this project was established at Changa Manga in the province of Punjab located at 80 km southwest of Lahore [13]. The current study was designed to estimate the health of the critically endangered WRVs in Changa Manga, Lahore, using a technique that does not require drawing blood.

2. Materials and Methods

2.1 Chemicals and Reagents

The chemicals used were absolute alcohol, acid alcohol, 3% malachite green and Kinyoun's carbol fuchsin. Acid alcohol was prepared using 10 ml of sulfuric acid with 90 ml of absolute alcohol and stored at room temperature. Three percent of malachite green was prepared by dissolving 3 g of malachite green in 100 ml of distilled water.

2.2 Study Population

The study population comprised WRVs from Changa Manga, Lahore (Fig. 1). Fourteen samples were collected from 7 pairs of vultures including 7 males and 7 females. Due to the limited availability of equipment and chemicals, only gross examination of feces and feathers, Gram staining of stool sample and fecal float method were performed.

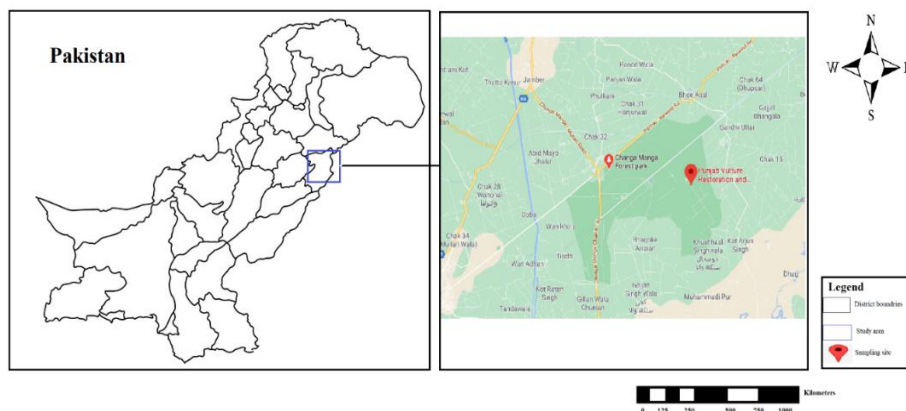


Figure 1. Map of the study area and the location of the sampling sites at Changa Manga, Pakistan

2.3 Fecal Collection

Fecal samples were collected without any contamination by spreading a polyvinyl sheet in the cage. Samples were collected in a clean container using the lid of bottles [14]. They were transported to the Virtual University of Pakistan, Lahore to check for endoparasites.

2.4 Fecal Sample Preparation

Different chemical tests were performed with the fecal samples. A fecal smear was prepared to check the presence of bacteria and other microorganisms, which is indicative of disease in the digestive tract. A small amount of fecal sample was examined under a microscope at 10× and 40× power. For oocytes of the coccidian species (*Cyclospora* and *Cyctoisospora*), a smear was prepared and dried at 60 °C, fixed with absolute alcohol for 30 seconds, stained with carbol fuchsin for one minute, and washed with distilled water. The slide

was then de-stained with acid alcohol for 2 minutes, washed, and dried on a slide warmer at 60 °C. It was observed under 40× or higher magnification. Fecal matter of seven pairs was examined and analyzed for protozoan or helminthes infection. Its gross examination involved the consistency of feces, color of feces, presence of blood in the feces and gross parasites.

3. Results

The samples were observed with the naked eye and then examined under the microscope. All samples had a different color and consistency but no blood was found in any of the fecal materials. Pair 1 was infested with oocytes; however, in pairs 5 and 6 only the male was infested and the female was infested in Pair 7. Pairs two, three and four were completely healthy (Table 1).

Table 1. Estimation of The Different Parameters of The Fecal Material

Pairs	Gender	Weight (Kg)	Age (Years)	Consistency	Color	Presence of blood	Gross parasites
1	♂	6.8	9	Solid, sandy	Dark brown, green	No	Visible
	♀	7.3	9.5	Solid	Brown, green	No	Visible
2	♂	7.5	8	Solid, sandy	Dark brown	No	Not visible
	♀	6.8	8.8	Semi solid	Black	No	Not visible
3	♂	6.7	8.5	Solid	Brown, black	No	Not visible
	♀	7.1	8	Solid, sandy	Brown	No	Not visible
4	♂	6.8	9	Solid	Brown	No	Not visible
	♀	6.7	10	Semi solid	Brown	No	Not visible
5	♂	5.8	11	Solid, sandy	Green	No	Visible
	♀	6.2	10.5	Solid, sandy	Black, light brown	No	Not visible
6	♂	7.1	9	Solid	Green	No	Visible
	♀	7.2	8.5	Solid	Dark brown	No	Not visible
7	♂	8	11.5	Solid	Black	No	Not visible
	♀	7.9	10	Semi solid	Brown, green	No	Visible

Gram staining was also performed to assess the presence or absence of bacteria in the digestive tract of WRVs. All the pairs were either gram-positive or gram-negative. Only Pair 5 was negative for all Gram staining (Table 2).

Table 2. Characterization of Gram Staining from the Feces of WRVs

Pairs	Gender	Gram +	Gram -
1	♂	-ve	-ve
	♀	-ve	+ve
2	♂	+ve	-ve
	♀	+ve	-ve
3	♂	-ve	-ve
	♀	-ve	+ve
4	♂	-ve	+ve
	♀	+ve	+ve
5	♂	-ve	-ve
	♀	-ve	-ve
6	♂	-ve	+ve
	♀	+ve	+ve
7	♂	-ve	-ve
	♀	+ve	+ve

-ve; negative and +ve; positive

4. Discussion

Endoparasites are organisms that live inside an animal's body and are harmful [15]. In order to check the health status of the WRVs, only limited fecal and feather samples were collected as the birds were in the middle of the breeding season. Gross examination of feces showed its consistency... Green color indicates the presence of parasites, dark brown indicates anoxia for about 24 hours and red color indicates the presence of blood, which shows infection in the intestines [16].

Several studies about intestinal parasites have been conducted on wild birds [17-22]. Our study revealed the presence of coccidiosis in the fecal samples [23]. Another study reported that coccidiosis is among the most frequent and common diseases of birds in the world. In almost 30 species of birds, the most common coccidiosis genus was *Cryptosporidium* spp which acts as an intestinal pathogen [24]. For the *Cryptosporidium* genus, three species including *C. meleagridis*, *C. baileyi* and *C. galli* cause renal and respiratory diseases in many bird species [25-27] which result in high mortality [28]. Kocijan et al. also studied the Eurasian griffon vultures' (*Gyps fulvus*) microflora and digestive tract parasites and concluded that parasitic findings depend on their feeding habits. [29].

The presence of microflora in the predator's intestine depends on its diet [16]. Houston and Cooper studied gram-negative bacilli especially *E. Coli* of family Enterobacteriaceae. They reported that these bacteria were normally found in the intestinal tract of the whiteback griffon vulture and some other members of the family Falconiformes because of the mostly intestinal flora derived from their prey [30]. Gram staining works on the principle that the organism's cell wall determines whether the organism is gram-negative or gram-positive. The bacteria which retain the crystal violet, which is the primary stain, are gram-positive. While the bacteria that are decolourized and then counterstained are gram-negative [31]. The bacteria isolated from the vultures can spread diseases to other wildlife and human populations, especially *E. coli* [30, 32]. The presence of bacteria, whether it's gram-positive or gram-negative, shows intestinal inflammation or irritation. To detect the parasites' eggs, a floatation solution that has a higher gravity than the eggs is used. Fecal floatation is a veterinary test used for the diagnosis of worms and

internal parasites. It helps in detecting the eggs of mature parasites present in the body. The eggs of these parasites are passed along with the feces of the host. In this test, egg floats on the surface and other heavy parts of the fecal sample sink towards the bottom [33]. The health status of WRVs is also measured by their age, weight and egg hatching ratio [34].

5. Conclusion

The current study revealed that although the presence of bacteria in the fecal material is normal but these bacteria can evolve into a pathogenic status under stress or due to a depressed immune system. The fecal samples indicated the presence of parasites in some pairs and almost all pairs were positive for gram-staining.

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